Chemotaxis assays

Special thanks to Prof. Eisenbach's lab, especially Oshri Afanzar.

Swarming

This technique serves for observation of chemotaxis but not for quantitative results. Also, spreading of the bacteria depends on the motility of the cells, metabolism and chemotaxis. Thus, the cells should be pre-examined for having good motility and ability to **metabolize the attractant**. Swarming can be carried out either on TB plate or minimal medium (H1) plate with semi-solid agar.

Preparation

For 4 Petri dishes

20ml 2xTB

For TB preparation protocol please see notes section.

20ml 0.6% Bacto-agar.

0.12gr agar powder with 20 ml of sterile– water (better make that 0.5%).

Heat the agar using a microwave till it dissolves (for unexperienced, autoclave is preferable)[1]

Heating plate.
4 Petri dishes.

Protocol:

1. Prepare overnight culture (inoculate from glycerol stock or single colony) in TB medium with appropriate antibiotics (if required) and grow cells overnight at 30 while shaking.
   A plate culture can be used as well.
2. Place Petri dishes onto the heating plate (it should be off) - **DO NOT MOVE OR TOUCH THE PLATE FROM THIS POINT ON**!
3. Mix 1:1 agar with the TB medium.
4. Pour immediately 10 ml into a Petri dish and leave it at room temperature until solidification.
5. Make **SURE** that the gel is not cracked.
6. Using a sterile tip, touch the overnight medium or a colony and simply touch the agar in the center.
7. Turn heating plate to **30°C** and incubate at for 3-5 h (time depends on strain) – the final result should be a ring in the agar.

[1] Using autoclave will assure that no contamination is present and that the agar has dissolved properly.

Chemical-in-plug assay

This assay is a qualitative one but, in contrast to swarm assay, it is not depending on metabolism.

Preparation

Overnight culture in TB.

Motility buffer

10mM KPi, pH 7, 0.1mM EDTA, 0.001 mM methionine

Whatman filters cut into small disks.

Protocol:

1. Prepare overnight culture (inoculate from glycerol stock or single colony) in TB medium with appropriate antibiotics (if required) and grow cells overnight at 30 while shaking
2. Dilute 1:50 overnight culture with TB.
3. Grow at 30°C to O.D.590 = 0.9.
4. **Devied into tubes with liquid height not more than 3 cm!**
5. Centrifuge **at 1000 (G**) for 10 minutes – the pellet should be soft.
6. Add motility buffer and centrifuge **at 800-1000 (G**) for 10 minutes – the pellet should be soft. **Again not more than 3 cm!**
7. Remove the buffer using Pasteur pipet.
8. Repeat step 3+4 two more times.
9. Add motility buffer, final O.D. should be 1.5-2.
10. Prepare an 0.5-0.3% agar with motility buffer.
11. Mix the cells at 1:1 with agar prepared with motility buffer (temp of the agar should not be above 37°C) final concentration of the cells and agar should be 0.75-1 at O.D.590 0.25-0.15% respectively.
12. Pour 10 ml of the cells with the agar into a Petri dish and let the agar solidify - **DO NOT MOVE OR TOUCH THE PLATE FROM THIS POINT ON**!
13. Make **SURE** that the agar is not broken!
14. Soak the Whatman disks using 20 microliters of the chemo-attractant/replant solution
15. Air-dry the disks – simply make sure the disks are not dripping with the solution.
16. Place up to 5 disks onto the agar. Mark each disk on the plate with appropriate info.
17. Add another motility soaked disk onto the center of the agar as control.
18. Incubate at **room temp,** or 30°C for several hours – (0.5 – 4 hrs).

Notes

TB preparation

Tryptone broth

- 10 g tryptone

- 5 g NaCl
- Add H2O to 1L and autoclave.